Genetic diversity of Ukrainian local pig breeds based on microsatellite markers

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Preserving the current diversity of the living material on Earth is fundamental for the survival of future generations . A study was conducted to investigate the genetic diversity of Ukrainian local pig breeds. A total of 350 pigs representing five local pig breeds from Ukraine (Mirgorod – MIR, Poltava Meat – PM, Ukrainian Meat – UM, Ukrainian White Steppe – UWS and Ukrainian Spotted Steppe – USS) and one commercial breed (Duroc, DUR) were sampled. Twelve microsatellite loci (*SW24*, *S0155*, *SW72*, *SW951*, *S0386*, *S0355*, *SW240*, *SW857*, *S0101*, *SW936*, *SW911* and *S0228*) were selected and belong to the list of microsatellite markers recommended by ISAG. The results indicate that there exists, in general, a high degree of genetic variability within the five Ukrainian local pig breeds. However, the genetic variability in the MIR and PM breeds was significantly lower (mean Na = 2.92–3.92; Ho = 0.382–0.411; F_{IS} = 0.178–0.184) than in the other three Ukrainian local pig breeds – UM, UWS and USS (mean Na = 5.00–8.42; Ho = 0.549–0.668; F_{IS} = 0.027–0.066). Thirty-four private alleles were identified among the six analyzed genetic groups which were distributed between 11 of the 12 loci. A high number of alleles typical for the breed (private alleles) was observed in Duroc pigs – 9 alleles did not occur in Ukrainian local pig breeds. The HWE test showed that all of the polymorphic loci deviated from HWE (P < 0.05) in at least one population. Loci *S0355* (5), *S0386* (4) and *SW24* (4) presented a higher number of populations in imbalance. The mean F_{ST} showed that approximately 77.8% of the genetic variation was within-population and 12.2% was across the populations. The five Ukrainian local breeds were classified into two major groups, according to the phylogenetic tree, which was based on standard genetic distance. Overall, we found that 92.6% of the individual pigs were correctly assigned (324 out of 350) to the respective breed of origin, which is likely a consequence of the well-defined breed structure. Probabilities from the allocation test of individuals for the six pig genetic groups were estimated with Structure Harvester. In cluster 1 the highest grouping probabilities were found for the MIR (0.917) and PM (0.750) breeds. Local breeds UM (0.824) and USS (0.772) were grouped in cluster 2. Cluster 3 was related to the local pig breed USW (0.873). Cluster 4 presented high allocation probabilities for the commercial pig breed Duroc (0.924). The obtained results are important for the future conservation of Ukrainian local pig breeds.

Keywords: STR loci; genetic characterization; swine; Ukraine

Introduction

Preserving the current diversity of the living material on Earth is fundamental for the survival of future generations. In the case of animals, in the recent past, more and more effective breeding programmes have been implemented, and have led to an emphasis on a few specialised stocks. Consequently, breeds that are less suited to current needs tend to see their numbers decline and to be eventually lost. Conservation of variation, however, is necessary to meet future agricultural challenges and particularly food needs, as well as to preserve the rich agricultural heritage of the various regions of the world (SanCristobal et al., 2003).

Livestock populations have been subjected to a variety of evolutionary forces during their histories. The cumulative effect of foundation events, genetic drift and natural or artificial selection has led to the formation of distinct breeds. In the process of evaluating genetic diversity to develop conservation programmes, it is of interest to assess genetic variation between domestic stocks by using powerful tools such as genetic markers (Berthouly et al., 2008).

At present there are more than 730 breeds or lines of pigs throughout the world and more than two thirds are found in China and Europe. Many of these (possibly more than 270 breeds) are now in danger of extinction and others are threatened by inefficient use or loss due to cross breeding. To evaluate genetic uniqueness and breed diversity of pigs and assist in rationalising breed conservation programmes, microsatellites have been and remain efficient markers (Nidup & Moran, 2011).

Europe contains a large proportion of the world population of pigs (circa 30%) as well as of the world genetic diversity of pigs (37% of the breeds included in the FAO inventory). However, the European pig industry relies predominantly on a limited number of breeds, since one single breed, the widely known Yorkshire (Large White in many countries), represents about one third of the slaughter pigs' gene pool of the European Union (Laval et al., 2000). Local breeds can be considered cultural properties in relation to their role as historical witnesses as they often play a central part in the agriculture tenures and in the social life of rural populations. Local breeds can also be likened to cultural properties because they contribute to the preservation of ancient local traditions. The analysis shows that consistent differences can be observed in the cultural values of local breeds, both as historical witness and as custodian, today, of local traditions (Gandini & Villa, 2003). It can be assumed that local breeds contain the genes and alleles per-

tinent to their adaptation to particular environments and local breeding goals. Such local breeds are needed to maintain genetic resources permitting adaptation to unforeseen breeding requirements in the future and can serve as a source of research material (Romanov & Weigend 2001).

Microsatellite markers (MS) also referred to as short tandem repeats (STR), short sequence repeats (SSR) or sequence tagged microsatellite sites (STMS) contain repetitive sequences composed of 2–6 nucleotides. The most common motif in the pig genome is $(CA)_n$ and its number is estimated to range between 65,000 and 100,000 copies (Winterø et al., 1992). Microsatellites have been proposed as the best markers for evaluating the genetic diversities of domestic animals because of their abundant, even distribution in the genome, high polymorphism and ease of genotyping. The International Society of Animal Genetics (ISAG) and the Food and Agriculture Organization (FAO) have recommended a set of 27 microsatellite loci (www.toulouse.inra.fr/lgc/pig/panel/html) for evaluating the genetic diversities of pigs as part of the global strategy for the management of farm animal genetic resources (Hammond & Leitch, 1998).

The analysis of the MS loci showed that the allelic diversity pattern among breeds was quasi-independent from the diversity pattern based on allele frequencies. Genetic distances showed no particular clustering of local with international breeds, confirming the genetic uniqueness of the European local breeds compared to mainstream international breeds (Ollivier, 2009). Within breeds of the same species (or within one species) it is possible to spot the ancient or the most estranged from the "protogene pool" groups of animals and, thus, to use these data in the breeding strategy aimed at the preservation of animal forms close to the ancestral form. Using various DNA markers, methods of genomic and genetic breeding in nature conservation and genetic selection of domesticated species provides an opportunity to obtain data on the genetic potential (value, originality) of the breed, which is important for scientific justifycation of its preservation (Stolpovskiy & Zakharov-Gezekhus, 2017).

Thus, the focus in this paper will be on the genetic diversity pigs of five Ukrainian local and one commercial (Duroc) breeds assessed by using genetic markers (microsatellite DNA loci).

Material and methods

This study was carried out to understand the molecular genetic diversity of six swine populations in Ukraine. A total of 350 pigs representing five local pig breeds from Ukraine (Mirgorod, MIR: n = 26; Poltava Meat, PM: n = 13; Ukrainian Meat, UM: n = 128; Ukrainian White Steppe, UWS: n = 67 and Ukrainian Spotted Steppe, USS: n = 25) and one commercial breed were sampled. The commercial breed pigs (Duroc, DUR: n = 91) were included in the investigation in order to complete the picture of the diversity existing in populations of pigs in different origins. The samples, for both sexes (sows and boars), were collected in five different region of Ukraine (Table 1).

Table 1
Sampling information
of five Ukrainian local and commercial pig breed

Breed	Code	Origin	Number of samples
Mirgorod	MIR	Poltava region	26
Poltava Meat	PM	Lugans'k region	13
Ukrainian Meat	UM	Kherson region	128
Ukrainian White Steppe	UWS	Kherson region	67
Ukrainian Spotted Steppe	USS	Kherson region	25
Duroc	DUR	Zaporizhzhya region, Mykolayiv region	91

PCR analysis was carried out on DNA extracted from 350 ethanol-fixed small tissue samples (pieces of ear). A DNA extraction using the Nexttec Clean Column kit (Nexttec, Leverkusen, Germany) was performed according to the manufacturer's instructions. Genomic DNA was extracted based on the Zinovieva and Gladyr's (2011) protocol, with minimum adaptations.

Twelve microsatellite loci (*SW24*, *S0155*, *SW72*, *SW951*, *S0386*, *S0355*, *SW240*, *SW857*, *S0101*, *SW936*, *SW911* and *S0228*) recom-

mended by the International Society for Animal Genetics (ISAG) were used to analyze the genetic diversity of six Ukrainian swine breeds. Electrophoresis was conducted using an ABI 3130xl Genetic Analyzer (Applied Biosystems, USA). The size of each allele was visualized and determined by GeneMapper version 4.0 software (Applied Biosystems, USA). The DNA of the samples were stored in the DNA Bank of the Federal Science Center for Animal Husbandry named after Academy Member L. K. Ernst where this experiment was developed.

In the analysis of the genetic variability within and between Ukrainian swine breeds, the GenAIEx version 6.5 software (Peakall & Smouse, 2012) was used to calculate population genetic parameters such as: allelic frequencies observed (*Ho*) and expected heterozygosity (*He*) for each locus. The effective allele number (*Ae*) for each swine breed or locus was calculated using the following formula:

$$Ae = 1 / (1 - He),$$

where *He* corresponds to the expected heterozygosity for each swine breed or locus, respectively.

We determined levels of genetic differentiation within and among six Ukrainian pig populations using the indices proposed by Weir & Cockerham (1984): F_{IT} (=*F*), F_{ST} (=*Θ*) and F_{IS} (=*f*) in FSTAT version 2.9.3 software (Goudet, 2002). The bootstrap values were obtained to estimate the statistical signifycance for each of the indices by permutation test (999 permutations).

GENEPOP version 4.2 software (Rousset, 2008) was used to find a significant deviations form Hardy-Weinberg equilibrium (HWE) per breed and locus using Markov chain algorithm (Guo, Thompson, 1992) with 10,000 dememorizations, 200 batches and 5,000 interactions per batch (the exact test).

The genetic relation between swine breeds was estimated based on Nei (1972) standard genetic distances using the GenAIEx version 6.5 software (Peakall & Smouse, 2012). A frequency-based population assignment-test (Paetkau et al., 1995) was carried out and the leave-one-out procedure was used the GenAIEx version 6.5 software (Peakall & Smouse, 2012).

A dendrogram (hierarchical tree diagram) was created using the UPGMA method (Unweighted Pair Group Method with Arithmetic Mean) (Sneath & Sokal, 1973). Bootstrap analyses (with 999 permutations) were used to estimate the internal consistency of the suggested groupings in the PAST version 3.01 software (Hummer et al., 2001). A Principal Coordinate Analysis (PCoA) was done to visualize the geometric relationships among the Ukrainian swine breeds using GenAlEx version 6.5 software (Peakall & Smouse, 2012).

Population genetic structure of the swine breeds was investigated using Pritchard et al. (2000) algorithm implemented in Structure version 2.2 application based on the multilocus microsatellite genotypes. The Structure software is able to determine genetically distinct clusters (populations of origin, *K*) of the sampled pigs.

Based on the Markov chain Monte Carlo method (MCMC), the Structure Harvester algorithm (Earl, 2012) was used to estimate the natural algorithm of the probability that any particular individual belongs to the assumed *K* clusters (Evanno et al., 2005). The program provides a fast way to assess the range of possible clusters (*K*) from 2 to 11, and was run 10 times for each *K*.

Results

All microsatellite loci exhibit substantial levels of polymorphism across the study samples. A total of 132 alleles were detected at these 12 marker loci in the 350 evaluated samples of Ukrainian pigs. Only the alleles observed once were not included in the analyses. The number of observed alleles (*Na*) detected per polymorphic microsatellite locus ranged from two (MIR, *S0355* and PM, *SW951*) to 14 (UM, *SW24*).

Shared alleles were detected in high frequencies among Ukrainian swine breeds in most of the studied loci (Table 2). Eight loci presented from 1 to 2 shared alleles in all Ukrainian local pig breeds: *S0155*, *SW72*, *SW951*, *S0386*, *S0355*, *SW240*, *SW857* and *S0101*. For the UM and UWS pig breeds higher total alleles (101 and 88, respectively) and rare alleles lower than 5% numbers (43 and 41, respectively) were detected across the 12 microsatellite loci analyzed. The Duroc popula-

tion had a lower allele number, with 80 alleles, from which 34 had frequency lower than 5%.

Table 2
List of the common alleles (with frequencies higher than 20%) observed among five Ukrainian local and one commercial pig breed (na – data not available)

Locus	Breed					
	MIR	PM	UM	UWS	USS	DUR
SW24	99	99, 107	107	107, 113	95, 107	101, 117
S0155	158	148, 158, 160	148, 160	158, 160	158, 160	154, 160
SW72	103, 111	103, 113	103,	103	103, 113	103, 113
SW951	120, 122	120	120, 122	120, 128	120	120, 126
S0386	174, 176	174, 176	174, 176	166, 174, 176	174, 176	176, 184
S0355	245, 249	245, 249	245, 247	245, 247	247, 259	245, 247
SW240	95	95	95	95	95	93, 95
SW857	147, 149	147, 149	147, 149	147, 151	139, 147, 149	149, 153
S0101	207, 211	209, 211	209, 213	209, 211	209	209
SW936	na	na	99, 111	111	99	105, 111, 113
SW911	na	na	159, 169	159, 165	159	157
S0228	na	na	256, 260	258, 276	258, 260	260

There exist population-specific alleles found in a single swine breed ('private alleles'). Thirty-four 'private alleles' were identified among all tested pig breeds (Table 3) which were distributed between 11 of the 12 loci. A high number of 'private alleles' was detected in the Duroc population – nine alleles did not occur in other Ukrainian pig breeds. In animals of the UM breed, a total of 16 'private alleles' were detected. The number of 'private alleles' discovered for other Ukrainian local pig breeds was lowest (1–4 only).

Table 3
List of the 'private alleles' (in bp) found among five Ukrainian local and one commercial pig breed

Locus	Breed					
	MIR	PM	UM	UWS	USS	DUR
SW24	97	–	105, 125, 131	121	–	–
S0155	–	–	–	–	–	–
SW72	–	117	99, 107	–	–	131
SW951	–	–	118	116, 136	–	132
S0386	172, 178	–	–	–	–	186
S0355	–	–	–	–	263	243
SW240	–	–	–	–	–	123
SW857	–	–	–	159	–	145
S0101	–	–	193, 215	–	–	–
SW936	–	–	89, 93, 117	–	–	–
SW911	–	–	161, 167, 175, 177	–	–	–
S0228	–	–	274	–	–	254, 266, 270
Total	3	1	16	4	1	9

The effective number of alleles (Ae) varied from 1.28 to 5.89 and was proportional to the values of expected heterozygosity (He) found in loci *SW951* for the USS breed (0.218) and *SW24* for the UM breed (0.830), respectively.

The highest heterozygosity (over 80%) was detected for loci *S0101* (MIR and PM breeds), *SW857* (MIR and PM), *SW72* (UM) and *SW24* (UWS and DUR). The lowest value (< 20%) was revealed for loci *S0355* and *SW24* in MIR and for locus *SW951* in PM.

The HWE test showed that all of the polymorphic microsatellite loci deviated from HWE (P < 0.05) in at least one population (Table 4). Loci *S0355* (5), *S0386* (4) and *SW24* (4) presented a higher number of populations in the HWE imbalance. As shown in Table 4, two (UM and UWS) of the five studied Ukrainian local swine breeds had deviated from the HWE for most loci. The Ukrainian local pig breeds presented from one (for USS) to seven (for UWS) loci that deviate from HWE, while the Duroc had eight loci that did not fit HWE (Table 4). This underlines a great difference in the number of loci in Hardy-Weinberg disequilibrium among the five Ukrainian local and one commercial pig breed (Table 4).

Table 4
Results of the HWE test for 12 microsatellite loci identified on five Ukrainian local and one commercial pig breed (D – deficit heterozygosity; E – excess heterozygosity; * – P < 0.05; ** – P < 0.01; *** – P < 0.001; ns – P > 0.05)

Locus	Breed					
	MIR	PM	UM	UWS	USS	DUR
SW24	D***	D***	D***	D**	ns	ns
S0155	ns	ns	D*	D**	ns	D**
SW72	ns	ns	ns	D*	ns	E**
SW951	D*	ns	ns	D***	ns	ns
S0386	D***	ns	D***	D***	ns	D***
S0355	D***	ns	D***	D***	D*	D***
SW240	ns	D*	D***	D*	ns	ns
SW857	ns	ns	ns	ns	ns	D*
S0101	E*	E*	D*	ns	ns	ns
SW936	–	–	ns	ns	ns	E*
SW911	–	–	D*	ns	ns	D**
S0228	–	–	ns	ns	ns	D**

According to the genetic diversity indices (Table 5), the mean allele number (Na) was lowest for PM (2.92 alleles/locus), while the UM and UWS populations presented highest estimates of 8.42 and 7.33, respectively. The total Na was 6.23 alleles/locus (ranging between two to 14), whereas only two (UM and UWS) of the Ukrainian local pig breeds and the Duroc presented Na above the total mean score.

Table 5
Estimates of genetic diversity indices for 12 microsatellite loci detected on five Ukrainian local and one commercial pig breed

Breed	Na	Na (95%)	Ae	Ho	He	F_{IS}
MIR	3.92 ± 0.81	3.25 ± 0.64	2.30 ± 0.44	0.411 ± 0.098	0.486 ± 0.089	0.178 ± 0.102
PM	2.92 ± 0.57	2.58 ± 0.51	2.08 ± 0.42	0.382 ± 0.096	0.452 ± 0.087	0.184 ± 0.099
UM	8.42 ± 0.74	4.83 ± 0.34	3.83 ± 0.29	0.668 ± 0.030	0.718 ± 0.027	0.066 ± 0.030
UWS	7.33 ± 0.63	3.92 ± 0.22	3.13 ± 0.28	0.587 ± 0.039	0.649 ± 0.033	0.089 ± 0.047
USS	5.00 ± 0.47	3.33 ± 0.25	2.65 ± 0.25	0.549 ± 0.046	0.574 ± 0.048	0.027 ± 0.042
DUR	6.67 ± 0.64	3.83 ± 0.29	3.07 ± 0.39	0.551 ± 0.062	0.616 ± 0.047	0.111 ± 0.070

Note: Na – number of different alleles; Na (95%) – number of different alleles with a frequency ≥5%; Ae – number of effective alleles; Ho – observed heterozygosity; He – expected heterozygosity; F_{IS} – inbreeding coefficient.

The average values of observed heterozygosity (Ho) in the studied swine populations varied from 0.382 ± 0.096 (for PM) to 0.668 ± 0.030 (for UM). For the PM, the effective number of alleles ($Ae = 2.08 ± 0.42$) and the expected heterozygosity ($He = 0.452 ± 0.087$) were the lowest values amongst all analyzed pig breeds. On the other hand, UM was the breed that presented the highest estimated values of effective number of alleles ($Ae = 3.83 ± 0.29$) and expected heterozygosity ($He = 0.718 ± 0.027$). Comparing the estimates of expected heterozygosity and the effective number of alleles, Duroc possesses a high amount of genetic diversity compared to all other studied pig breeds, with the exception of UM and UWS.

The variability within pig breeds estimated using the inbreeding coefficient (F_{IS}) showed that the highest value was observed for the PM and MIR breeds (0.184 ± 0.099 and 0.178 ± 0.102, respectively). In the

local swine breeds it can be inferred that significant and positive F_{IS} values are a consequence of the inbreeding effect, arising from matings between related sows and boars. The Duroc presented a high significant inbreeding value also ($F_{IS} = 0.111 ± 0.070$). The high positive values for F_{IS} in five pig breeds also showed a significant deviation from HWE (Table 4). The fixation coefficient of populations (F_{ST}) per locus varied from 0.057 (*SW72*) to 0.206 (*S0101*), with a mean value of 0.122 ± 0.010. Thus, 12.2% of the total genetic variation was explained by differences between pig populations (Table 6). For the F_{ST}-index, six of the loci (50.0%) presented values outside the 95% confidence interval, with four loci indicating significant values to determine differences between studied pig breeds.

The mean F_{IT} and F_{IS} values for all microsatellite loci were 0.204 and 0.092 with 41.7% and 50.0% of the values outside the 95% confi-

dence interval, respectively. The mean fixation index within the populations (F_{IS}) in each locus represented positive values, except for loci *SW72* (–0.086), *SW857* (–0.039), *S0101* (–0.035) and *SW936* (–0.050), which presented negative F_{IS}, indicating an excess of heterozygous (primarily for Duroc, MIR and PM). The positive F_{IS} (0.092 ± 0.039) for all loci of pig breeds reflects the deviations from the HWE for most Ukrainian local pig breeds (Table 4).

Table 6
F statistical estimates for 12 microsatellite loci identified on five Ukrainian local and one commercial pig breed

Locus	$f (=F_{IS})$	$\Theta (=F_{ST})$	$F (=F_{IT})$
SW24	0.096	0.088	0.176
S0155	0.122	0.136#	0.242
SW72	-0.086	0.057#	-0.024
SW951	0.054	0.145#	0.191
S0386	0.282	0.108	0.359
S0355	0.377	0.115	0.449
SW240	0.138	0.085	0.211
SW857	-0.039	0.125	0.091
S0101	-0.035	0.206	0.178
SW936	-0.050	0.135	0.092
SW911	0.108	0.131#	0.225
S0228	0.095	0.152	0.233
$\overline{X} \pm S\overline{x}$	0.092 ± 0.039	0.122 ± 0.010	0.204 ± 0.035
95% CI	[0.021; 0.170]	[0.104; 0.143]	[0.139; 0.271]

Note: # – significant values for F_{ST}.

The genetic distances (D_A) between each pair of pig breeds are shown in Table 7. The highest Nei's D_A value (0.824) was found between the MIR and Duroc breeds, while the UM and the USS breeds were quite close to each other (0.159). Nei's D_A value indicated that the UM was the Ukraine local pig breed closest to the commercial Duroc breed (0.268), and that the MIR was the most genetically distant from Duroc (0.824). Overall, the Duroc breed revealed the longest distances in relation to all Ukrainian local pig breeds, probably due to the geographic isolation, drift and/or sampling effects. This finding supports the fact that the evolutionary path of Ukrainian local pig breeds is totally different from the evolutionary path of commercial pig breeds.

Table 7
Genetic distances (D_A) estimated among six pig populations

Breed	MIR	PM	UM	UWS	USS	DUR
MIR	–					
PM	0.169	–				
UM	0.506	0.306	–			
UWS	0.491	0.417	0.270	–		
USS	0.638	0.419	0.159	0.325	–	
DUR	0.824	0.584	0.268	0.566	0.374	–

The first axis of the PCoA plot of the six breeds (Fig. 1) clearly distinguishes the MIR and PM from the other breeds, which indicates that the Nei's D_A values among the MIR and PM, on the one hand, and the other Ukrainian local pig breeds, on the other hand, is high.

Figure 2 presents a dendrogram built with the UPGMA method from Nei's (1972) D_A matrix. Two different groups were observed: the first one (with 55% confidence) formed by Duroc and three local pig breeds (UM, USS and UWS); and the second group (with 67% confidence) formed by the MIR and PM breeds (Fig. 1 also).

An assignment-test was used to investigate relationships between six pig breeds (Table 8). The percentage of individuals assigned to the breed of origin varied from 69.2 (for PM) to 100% (for USS). Generally, we found that 92.6% of the individuals (324 out of 350) were correctly assigned to the respective breed of origin, which is probably a consequence of the clear intrabreed structure. The individuals of the Duroc breed were assigned to its true population of origin with a frequency of 92.6%. Only few individuals had a high probability of mixed ancestry (e.g., two MIR individuals clustered together with PM and three PM with MIR; five UM were assigned to UWS and four to USS). Thus, the assignment-test results yielded further evidence for genetic originality of the five Ukrainian local pig breeds.
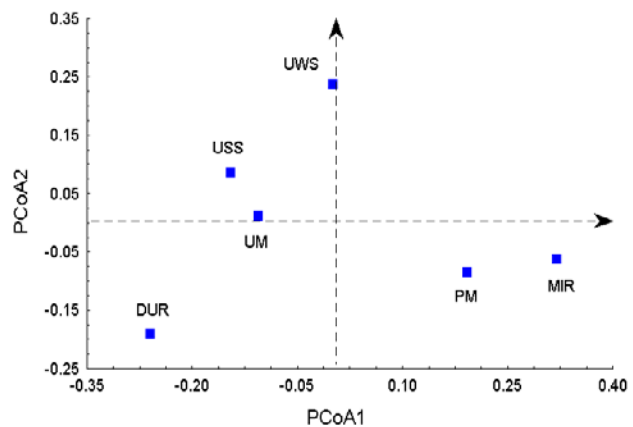

Fig. 1. Principal coordinate analysis (PCoA) plot for six pig populations based on Nei's genetic distances (D_A) using 12 microsatellite loci
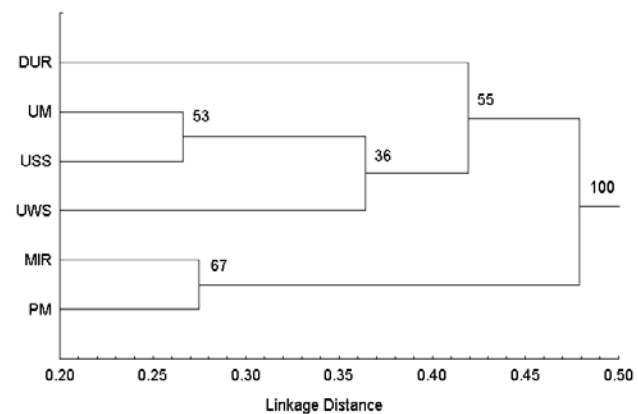

Fig. 2. UPGMA tree based on Nei's D_A genetic distance (Numbers indicate the proportion of bootstrap replicates sharing the labeled node)

The Bayesian approach implemented by Structure Harvester was used to evaluate the most likely number of ancestral populations underlying the observed interbreed genetic diversity. The likelihood of the observed data given the number of putative population of origin [Ln Pr(X/K)] is shown in Figure 3, for numbers of inferred populations (K) ranging from 2 to 11. The mean value of Ln Pr(X/K) increased up to $K = 4$ and then descended, with a large increase in its variation. Thus, it was assumed that $K = 4$ is the optimal number of clusters (ancestral populations) for the pig populations studied (Fig. 4).

Probabilities from the allocation test of individuals for the six pig breeds estimated using Structure Harvester (for $K = 4$) are presented in Table 9. In cluster 1 the highest grouping probabilities were revealed for MIR (0.917) and PM (0.750) breeds. Local breeds UM (0.824) and USS (0.772) were grouped in cluster 2. Cluster 3 was related to the local breed UWS (0.873). Cluster 4 indicated high allocation probabilities for the commercial pig breed Duroc (0.924). It is noteworthy that, all pig populations studied, despite having presented higher proportions in certain of the clusters, had several individuals allocated in other clusters.

Table 8
Assignment analysis of the five Ukrainian local and one commercial pig breed (values are the number of individuals from each breed (rows) assigned to each population (columns); italic indicates correct assignment. Accurate – the proportions of individuals derived from their source population)

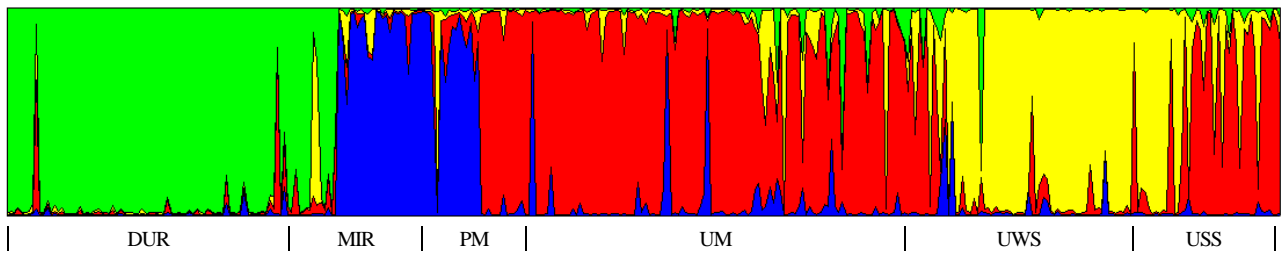| Breeds of origin	Breeds, based on n results of the assignment-test						Accurate., %
	MIR	PM	UM	UWS	USS	DUR	
MIR	*23*	2	1	–	–	–	88.5
PM	3	*9*	–	1	–	–	69.2
UM	–	1	*117*	5	4	1	91.4
UWS	–	–	3	*62*	1	1	92.5
USS	–	–	–	–	*25*	–	100.0
DUR	–	–	–	2	1	*88*	92.6

Fig. 4. Individual membership of the five Ukrainian local and one commercial pig breed of the *K* clusters (*K* = 4) inferred by Structure analysis: codes on the x-axis indicate the putative population of origin; see Table 1 for location abbreviations; each colour denotes a cluster from the structure analysis

Discussion

This is the first attempt to specifically quantify the population genetic diversity of the local Ukrainian pig breeds based on microsatellite markers. The six pig populations considered in our study showed substantial genetic diversity, with an overall mean of 6.23 alleles per locus (from two to 14) and an average expected heterozygosity of 0.572 for the 12 STR loci. At the breed level, the mean number of alleles per locus was 5.71 (2.92–8.42), with an allelic richness corrected for sample size of 2.84 (2.58–4.83) and an expected heterozygosity of about 0.525 (0.382–0.668). These results are in agreement with those observed for certain European pig breeds, but are somewhat lower than those reported for Asian breeds (Nidup & Moran, 2011).
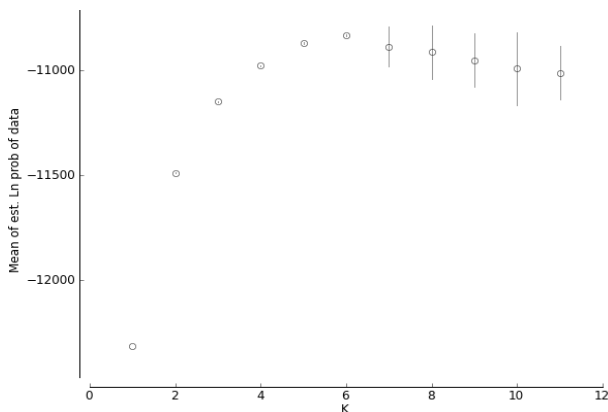

Fig. 3. Plot of mean likelihood *L*(*K*) and standard deviation (*SD*) per *K* value from Structure Harvester on a dataset containing 350 individuals of the five Ukrainian local and one commercial pig breed genotyped for 12 polymorphic microsatellite loci

Table 9
Allocation probabilities of individuals in five Ukrainian local and one commercial pig breed based on probabilities estimated with the Structure software (for *K* = 4)

Breed	Cluster 1	Cluster 2	Cluster 3	Cluster 4
MIR	0.917	0.033	0.039	0.011
PM	0.750	0.137	0.098	0.015
UM	0.056	0.824	0.080	0.039
UWS	0.027	0.079	0.873	0.021
USS	0.013	0.772	0.184	0.031
DUR	0.010	0.038	0.029	0.924

The mean number of alleles per locus of 5.00–8.42 recorded in the present study for the UM, UWS and USS pig breeds is more than twice the mean number of alleles of 2.92–3.92 recorded for the MIR and PM pig breeds, indicating higher genetic diversity in some local pigs of Ukraine but approximately corresponds to the mean 7.00–7.70 reported by Behl et al. (2002) for two Indian pig breeds.

The relatively high number of alleles found in the UM and USS pig breeds is an indication that the effects of isolation and artificial selection of these populations has been moderate. The lower number of alleles in the Duroc population (6.67) reflects a relatively recently established Ukrainian population of limited size. The number of alleles in the

Ukrainian Duroc pigs is, however, still higher than values of 2.39–2.80 reported in Belgian and certain Asian swine populations (Van Zeveren et al., 1995; Fan et al., 2002; Chang et al., 2009).

Thus, the PM breed, based on estimates of effective number of alleles and observed heterozygosity, can be regarded as the Ukrainian local pig breed with lowest genetic diversity. As previously mentioned in Niang Megha pig (Indian local breed), the low effective number of alleles may be due to very low frequency of most of the alleles at each locus and a very few alleles might have contributed the major part of the allelic frequency at each locus (Zaman et al., 2013). Overall, the three Ukrainian local pig breeds presented higher mean values for the intra-population genetic diversity parameters (such as mean estimation of *Na*, total allele and rare allele numbers), than the ones obtained for the commercial pig breed (Duroc). Additionally, the high amount of 'private alleles' found, mainly in the local swine breeds, shows their importance and the necessity of their preservation and conservation.

Such remark shows the high diversity of the local pig populations in comparison to the specialized (commercial) ones. This higher diversity may be explained by the fact that the locally adapted genetic groups are not subject to constant improvement programs for specific characteristics such as specialized breeds (Silva et al., 2011). Factors such as the level of inbreeding, population size, the history or origin of the breeding population, the level of artificial selection pressure and husbandry practices affect the genetic diversity of domestic animal populations (Ayizanga et al., 2016).

There was a significant level of inbreeding recorded at all loci studied. The lack of compliance with the HWE that was observed for at least one locus in most Ukrainian pig breeds is probably associated with the substantial deficit in heterozygosity. This shortage could be a consequence of inbreeding or intrabreed substructure, which are common properties in local breeds of low census population size (Ollivier et al., 2005; SanCristobal et al., 2006).

Moreover, in the analysed pig breeds, the high deviation from HWE loci number is probably due to the fact that the animals were raised by various pig breeding farms in Ukraine. These breeding farms often carry out matings between related individuals, especially due to the small size of the herd. On second hand, studying European commercial pig breeds, Laval et al. (2000) reported that the majority of them remained within HWE.

The mean overall fixation index (F_{IT}) of 20.4% recorded in the present study shows a great deal of genetic differentiation in individual animals relative to the total population. This index combines the genetic effects of non-random mating within populations together with the effects of genetic drift among populations (Ayizanga et al., 2016).

F_{ST} was the smallest at *SW240* and *SW24* loci (0.085–0.088) while at *SW951* locus it was the greatest (0.145). A mean F_{ST} of 0.122 indicates that 12.2% of total genetic variability occurs among the Ukrainian local pig populations and this is indicative of moderate genetic differentiation. This is comparable with results of Ayizanga et al. (2016) who reported a significant F_{ST} value of 12% among the local pigs of Ghana. AMOVA results of five Brazilian genetic groups (local and Landrace) obtained by Sollero et al. (2009) showed that 14% of all observed diversity came from the difference between the evaluated genetic groups. In another study carried out with Chinese pigs, an F_{ST} value of 7.7% was found (Yang et al., 2003). The highest genetic differentiation values for pig populations ever reported were F_{ST} = 27% (Laval et al., 2000)

between European pigs, followed by 26.1% for a differentiation study also carried out with European, Korean and Chinese pigs (Kim et al., 2005).

Results from the assignment test suggested a true genetic structure with significant differentiation among all populations, except the MIR and PM breeds. This result supports the trend from frequency-based D_A values, which showed significant differentiation among all population pairs but with the lowest DA observed between the MIR and PM breeds (0.169) and between the UM and USS breeds (0.159). Five populations displayed a pattern of strong distinctiveness, with more than 88% of individuals assigned to a single cluster in each case. By contrast, significant numbers of pigs from the PM breed were distributed over several clusters, with the most prominent cluster containing only 69.2% (9 from 13) of individuals. Overall, the results from the assignment test support the hypothesis of high homogeneity within most Ukrainian local pig breeds sampled (Traspov et al., 2016).

Conclusions

This study was the first based on microsatellite markers for the genetic characterization of the local pig breeds from Ukraine. The results show that levels of genetic diversity in five local Ukrainian pig breeds are moderate to high. Results from an assignment-test confirmed results from F_{ST} which suggested an original genetic structure with significant differentiation between most breeds sampled, but with little differentiation among MIR and PM breeds.

References

Behl, R., Kaul, R., Sheoran, N., Behl, J., Tantia, M. S., & Vijh, R. K. (2002). Genetic identity of two Indian pig types using microsatellite markers. Animal Genetics, 33(2), 158–159.

Berthouly, C., Bed'Hom, B., Tixier-Boichard, M., Chen, C. F., Lee, Y. P., Laloë, D., Legros, H., Verrier, E., & Rognon, X. (2008). Using molecular markers and multivariate methods to study the genetic diversity of local European and Asian chicken breeds. Animal Genetics, 39, 121–129.

Chang, W. H., Chu, H. P., Jiang, Y. N., Li, S. H., Wang, Y., Chen, C. H., Chen, K. J., Lin, C. Y., & Ju, Y. T. (2009). Genetic variation and phylogenetics of Lanyu and exotic pig breeds in Taiwan analyzed by nineteen microsatellite markers. Journal of Animal Science, 87(1), 1–8.

Earl, D. A. (2012). Structure harvester: A website and program for visualizing Structure output and implementing the Evanno method. Conservation Genetics Resources, 4(2), 359–361.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software Structure: A simulation study. Molecular Ecology, 14(8), 2611–2620.

Fan, B., Wang, Z.-G., Li, Y.-J., Zhao, X.-L., Liu, B., Zhao, S.-H., Yu, M., Li, M.-H., Chen, S.-L., Xiong, T.-A., & Li, K. (2002). Genetic variation analysis within and among Chinese indigenous swine populations using microsatellite markers. Animal Genetics, 33, 422–427.

Gandini, G. C., & Villa, E. (2003). Analysis of the cultural value of local livestock breeds: A methodology. Journal of Animal Breeding and Genetics, 120(1), 1–11.

Guo, S. W., & Thompson, E. A. (1992). Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics, 361–372.

Hammer, H., Harper, D. A. T., & Ryan, P. D. (2001). Past: Paleontological Statistics software package for education and data analysis. Palaeontologia Electronica, 4(1), 1–9.

Hammond, K., & Leitch, H. (1998). Implementing the global strategy for the management of farm animal genetic resources. FAO, Rome, Italy.

Kim, T. H., Kim, K. S., Choi, B. H., Yoon, D. H., Jang, G. W., Lee, K. T., Chung, H. Y., Lee, H. Y., Park, H. S., & Lee, J. W. (2005). Genetic structure of pig breeds from Korea and China using microsatellite loci analysis. Journal of Animal Science, 83(10), 2255–2263.

Laval, G., Iannuccelli, N., Legault, C., Milan, D., Groenen, M. A., Giuffra, E., Andersson, L., Nissen, P. H., Jørgensen, C. D., Beeckmann, P., Geldermann, H., Foulley, J.-L., Chevalet, C., & Geldermann, H. (2000). Genetic diversity of eleven European pig breeds. Genetics Selection Evolution, 32(2), 187.

Nei, M. (1972). Genetic distance between populations. The American Naturalist, 106(949), 283–292.

Nidup, K., & Moran, C. (2011). Genetic diversity of domestic pigs as revealed by microsatellites: A mini-review. Genomics and Quantitative Genetics, 2, 5–18.

Ollivier, L. (2009). European pig genetic diversity: A mini-review. Animal, 3(7), 915–924.

Ollivier, L., Alderson, L., Gandini, G. C., Foulley, J. L., Haley, C. S., Joosten, R., Rattink, A. P., Harlizius, B., Groenen, M. A. M., Amigues, Y., Boscher, M. Y., Russell, G., Law, A., Davoli, R., Russo, V., Matassino, D., Désautés, C., Fimland, E., Bagga, M., Delgado, J. V., Vega-Pla, J. L., Martinez, A. M., Ramos, A. M., Glodek, P., Meyer, J. N., Plastow, G. S., Siggens, K. W., Archibald, A. L., Milan, D., San Cristobal, M., Laval, G., Hammond, K., Cardellino, R., & Chevalet, C. (2005). An assessment of European pig diversity using molecular markers: Partitioning of diversity among breeds. Conservation Genetics, 6(5), 729–741.

Paetkau, D., Calvert, W., Stirling, I., & Strobeck, C. (1995). Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology, 4(3), 347–354.

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and researchd – an update. Bioinformatics, 28(19), 2537–2539.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. Genetics, 155(2), 945–959.

Romanov, M. N., & Weigend, S. (2001). Analysis of genetic relationships between various populations of domestic and jungle fowl using microsatellite markers. Poultry Science, 80(8), 1057–1063.

Rousset, F. (2008). Genepop'007: A complete re-implementation of the Genepop software for Windows and Linux. Molecular Ecology Resources, 8(1), 103–106.

SanCristobal, M., Chevalet, C., Foulley, J. L., & Ollivier, L. (2003). Some methods for analysing genetic marker data in a biodiversity setting – Example of the PigBioDiv data. Archivos de Zootecnia, 52(198), 173–183.

SanCristobal, M., Chevalet, C., Haley, C. S., Joosten, R., Rattink, A. P., Harlizius, B., Groenen, M. A. M., Amigues, Y., Boscher, M.-Y., Russell, G., Law, A., Davoli, R., Russo, V., Désautés, C., Alderson, L., Fimland, E., Bagga, M., Delgado, J. V., Vega-Pla, J. L., Martinez, A. M., Ramos, M., Glodek, P., Meyer, J. N., Gandini, G. C., Matassino, D., Plastow, G. S., Siggens, K. W., Laval, G., Archibald, A. L., Milan, D., Hammond, K., & Cardellino, R. (2006). Genetic diversity within and between European pig breeds using microsatellite markers. Animal Genetics, 37, 189–198.

Silva, E. C. D., Dutra Junior, W. M., Ianella, P., Gomes Filho, M. A., Oliveira, C. J. P. D., Ferreira, D. N. D. M., Caetano, A. R., & Paiva, S. R. (2011). Patterns of genetic diversity of local pig populations in the State of Pernambuco, Brazil. Revista Brasileira de Zootecnia, 40(8), 1691–1699.

Sneath, P. H., & Sokal, R. R. (1973). Numerical taxonomy. The principles and practice of numerical classification. W. H. Freeman and Company, San Francisco.

Sollero, B. P., Paiva, S. R., Faria, D. A., Guimarães, S. E. F., Castro, S. T. R., Egito, A. A., Albuquerque, M. S. M., Piovezan, U., Bertani, G. R., & Mariante, A. D. S. (2009). Genetic diversity of Brazilian pig breeds evidenced by microsatellite markers. Livestock Science, 123(1), 8–15.

Stolpovskiy, Y. A., & Zakharov-Gezekhus, I. A. (2017). The problem of conservation of gene pools of domesticated animals. Vavilov Journal of Genetics and Breeding, 21(4), 477–486.

Traspov, A., Deng, W., Kostyunina, O., Ji, J., Shatokhin, K., Lugovoy, S., Zinivieva, B., Yang, B., & Huang, L. (2016). Population structure and genome characterization of local pig breeds in Russia, Belorussia, Kazakhstan and Ukraine. Genetics Selection Evolution, 48(1), 16.

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. Evolution, 38(6), 1358–1370.

Wintero, A. K., Fredholm, M., & Thomsen, P. D. (1992). Variable (dG-dT)n (dC-dA)n sequences in the porcine genome. Genomics, 12(2), 281–288.

Yang, S. L., Wang, Z. G., Liu, B., Zhang, G. X., Zhao, S. H., Yu, M., Fan, B., Li, M. H., Xiong, T. A., & Li, K. (2003). Genetic variation and relationships of eighteen Chinese indigenous pig breeds. Genetics Selection Evolution, 35(7), 657.

Zaman, G., Shekar, M. C., & Aziz, A. (2013). Molecular characterization of Meghalaya Local pigs (Niang Megha) using microsatellite markers. Indian Journal of Science and Technology, 6(10), 5302–5306.

Zeveren, A. V., Peelman, L., Weghe, A. V. D., & Bouquet, Y. (1995). A genetic study of four Belgian pig populations by means of seven microsatellite loci. Journal of Animal Breeding and Genetics, 112, 191–204.

Zinovieva, N. A., & Gladyr, E. A. (2011). Geneticheskaya ekspertiza sel'skokhozyaystvennykh zhivotnykh: Primenenie test-sistem na osnove mikrosatellitov [Genetic evaluation of agricultural animals: The use of microsatellite test system]. Dostizheniya Nauki i Tekhniki APK, 9, 19–20 (in Russian).